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Letter to the Editor: Sequence-specific chemical shift assignment of calcium-loaded murine S100A4

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Biological context

S100A4, also known as Mts1, has been linked to metastasis through several lines of evidence (Ebralidze et al., 1989; Davies et al., 1993; Grigorian et al., 1993; for reviews see Barraclough, 1998; Sherbet and Lakshmi, 1998). Mts1 expression levels are higher in metastatic than in non-metastatic tumors, and are also high in cells with naturally high motility. Introduction of Mts1 can increase the metastatic character of cancer cells, while antisense ribozyme techniques targeted to Mts1 can reduce metastasis. Cross-breeding of mice engineered to express high levels of Mts1 with mice highly susceptible to non-metastatic tumors results in offspring with more aggressive tumors. Mts1 belongs to the S100 protein family, most members of which contain two calcium-coordinating EF hands. Calcium binding triggers a conformational rearrangement which can alter surfaces required for interaction with other proteins. Here, we present the sequential heteronuclear chemical shift assignments of Mts1 in its calcium-loaded form.

Methods and experiments

The H-MBP-3C plasmid (Alexandrov et al., 2001) was used to express and purify Mts1 as described previously (Dutta et al., 2002). After removal of the six histidine and MBP tags, the resulting protein contains 106 amino acids, representing the 101 amino acids of full length murine Mts1 preceded by five additional residues numbered −5 to −1 (there is no residue zero). Double labeled samples were created using M9 minimal media with $^{15}NH_4Cl$ and ^{13}C-glucose as the sole nitrogen and carbon sources. NMR samples contained 2.5 mM Mts1, 5 mM Tris-HCl, 20–25 mM $CaCl_2$, 1–3 mM EDTA, 10 mM d_{12}-dithiothreitol, 50 μM NaN_3 and 5% (v/v) D_2O at pH 6.0. NMR experiments were recorded at 40 °C using a Varian INOVA 600 MHz spectrometer with four rf channels and a triple-axis pulsed field gradient probe. VNMR (Varian) and nmrPipe (Delaglio et al., 1995) were used for data processing, and analysis was performed with NMRview (Johnson and Blevins, 1994). The backbone chemical shift assignment procedure utilized ^1H,^{15}N-HSQC, HNCO, HNCACB, CBCA(CO)NH and HBHA(CO)NH experiments, with side chain assignments and confirmation of residue types via analysis of H(CO)NH and C(CO)NH (Figure 1) TOCSY spectra.

Extent of assignments and data deposition

With the exception of the carbonyl carbons immediately preceding proline residues and the extreme C-terminal carbonyl carbon, 90% of the backbone resonances (HN, N, Cα, Cβ, C-carbonyl) were assigned. Multiple assignments were obtained for residue −4 through +3 and residues 98 to 99, indicating multiple stable conformations at the termini. Residues −4, +4 and 98 are proline, suggesting that cis-trans isomerization may contribute to the multiplicity. The resonances of 7 residues, including P43, S44 and five residues from a region which has been assigned to helix IV in apo-Mts1 (Dutta et al., 2002) were unobservable, presumably due to exchange broadening.

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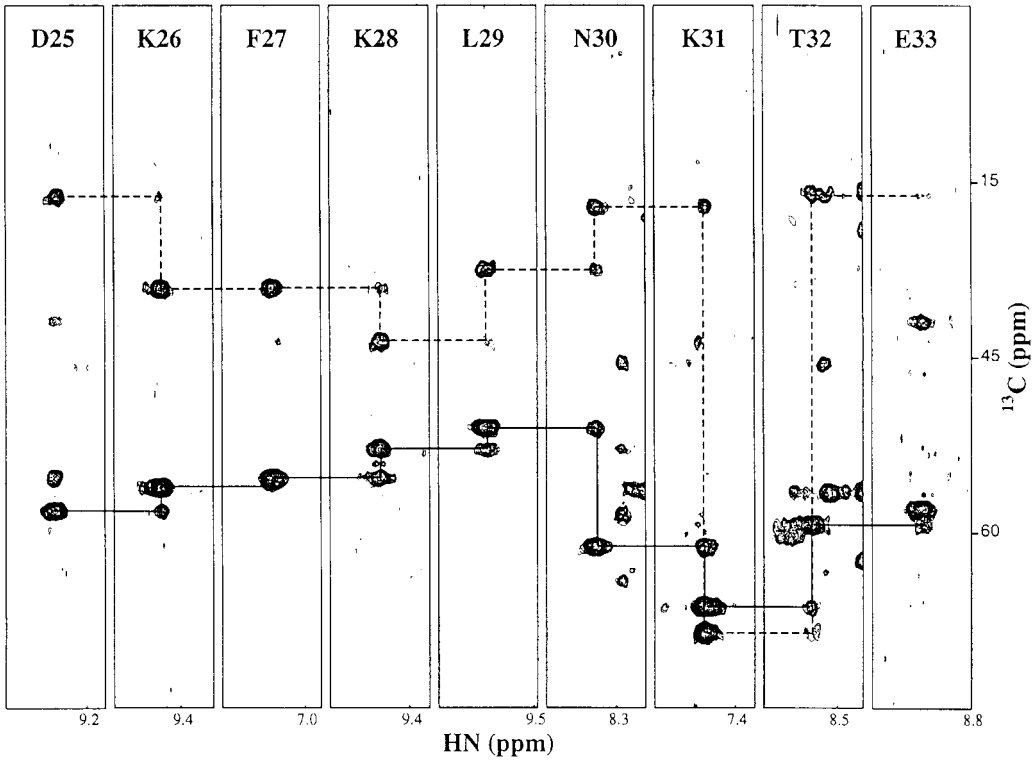

Figure 1. HN-N strip plots of HNCαCβ data set illustrating the quality of the data used to assign calcium-loaded murine Mts1.

Analysis of an ^{15}N-HSQC spectrum of an additional sample labeled only with ^{15}N-Cys confirmed the positions of the assigned C86 and C93 resonances, but did not produce additional peaks for the missing C76 and C81 resonances from helix IV. The inability to assign much of helix IV is not merely due to amide exchange, as amide resonances from the floppy termini are observable, and amide exchange did not interfere with assignment of apo-Mts1 resonances under similar conditions (Dutta et al., 2002). The suggested intermediate time-scale dynamics in this region could hint at a transient disruption or reorientation of helix IV in the calcium-bound state.

In total, 908 chemical shifts are reported, including 861 unique assignments and 47 extra resonances for the multiply assigned residues near the N and C-termini. The 861 unique assignments represent 73% of all resonances, and 84% of resonances excluding aromatic/guanidino/imidazole group side chains. Chemical shifts have been deposited in the BioMagResBank Database (accession code BMRB-5247). An analysis of chemical shift changes upon titration will be presented elsewhere.

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